David G. Stevenson[a]
Russell K. Doorenbos[b]
Jay-lin Jane[c]
George E. Inglett[a]

[a] Cereal Products and Food Science Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, IL, USA
[b] Department of Agronomy, Iowa State University, Ames, IA, USA
[c] Department of Food Science & Human Nutrition, Iowa State University, Ames, IA, USA

Structures and Functional Properties of Starch From Seeds of Three Soybean (*Glycine max* (L.) Merr.) Varieties*

Structures and functional properties of starch from high-protein, lipoxygenase-free and low-linolenic acid soybean variety seeds collected 20 d prior to harvest were investigated. Soybean starches exhibit C_B-type X-ray diffraction patterns, and granule diameters were very small (0.7 to 4 μm). Soybeans, 20 d prior to harvest contained 10.9–11.7% starch (dry basis). Apparent amylose content was 19–22% and absolute amylose content was 11.8–16.2%. Amylopectin weight-average molar mass ranged from 5.1 to 11.3×10^8 g/mol. Amylopectin average branch chain-length, determined by anion-exchange chromatography with an amyloglucosidase post-column and pulsed amperometric detector, was very short relative to other starches (20.4–20.9). Onset gelatinization temperature ranged from 52–54°C, and ΔH was 12–13 J/g. Paste viscosity was low relative to other starches, especially peak (81–93 RVU) and final (93–106 RVU) viscosity. The apparent amylose content of the low-linolenic acid soybean starch was significantly higher than that of high-protein soybean starch, and absolute amylose content of low-linolenic acid soybean starch was significantly higher than that of lipoxygenase-free soybean starch. Based on our results, investigations on whether soybeans with different fatty acid oil composition have different starch structures would be worthwhile. Field replicates for each soybean variety exhibited high variation in starch characteristics, with further differences in starch structures and functional properties likely to be determined once variation is minimized.

Keywords: Soybean; Starch structure; Starch function; Amylose; Amylopectin; *Glycine max*; Edamame

1 Introduction

Soybean is an extremely valuable crop to the economy of the United States of America. In 2003, USA planted 29.7×10^6 hectares (73.2×10^6 acres) of soybeans, produced 65.81×10^6 t ($2\,418 \times 10^9$ bushels), which generated revenues of US $\$18.5 \times 10^9$ [1]. Soybeans have a wide spectrum of uses including animal feed, dietary supplements, pharmaceuticals, emulsifiers, stabilizers, biodiesel fuel, engine oils, pesticides, disinfectants, antibiotics, lubricants, adhesives, insulation, printing inks and solvents [1].

Soybeans cultivated in Illinois, Indiana and Ontario, Canada have been reported to be composed of 43–48% protein, 18–21% oil, 4.9–6.8% sucrose, 0.8–1.2% raffinose and 3.5–4.3% stachyose [2–4]. Soybean seed protein and oil are nutritionally and economically important, with remaining components, except isoflavones and saponins, frequently regarded as undesirable. Starch content of mature soybeans is very low (0.19–0.91%) [5], and like peanuts [6], soybeans have the distinction of legumes that lack considerable levels of starch in their seeds at maturity. However, starch does accumulate in soybean seeds during development, and the highest level is found about 20 d prior to harvest with 10–15% of the dry matter [7]. Soybeans cultivated under elevated temperature and carbon dioxide levels have been reported to have 9–20% of their dry matter as starch at maturity [8] and elevated temperatures have been reported to inhibit starch degradation in peanuts [9]. The reasons why soybeans degrade the majority of their starch late in maturity, and whether the carbon is converted to protein or oil and influences their structures, remain unknown.

Starch is the main carbohydrate of plant storage organs. Starch has been extensively characterized in many cereal, root and tuber crops, as well as many legumes, but has not been studied in leguminous soybeans because of its low content at maturity. The only research on soybean starch

Correspondence: *David G. Stevenson*, Cereal Products and Food Science Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, 1815 N. University Street, Peoria, IL 61604, USA. Phone: +1–309–681–6447, Fax +1–309–681–6685, e-mail: stevensond@ncaur.usda.gov.

* Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

focused on starch content [5] or granule morphology [10]. In this study we characterize structure and properties of starch isolated from one variety each of high-protein, lipoxygenase-free and low-linolenic acid soybeans collected from seeds about 20 d prior to harvest. Characterization of these starches may provide insight of whether starch influences composition of mature soybeans. Additionally, characterization of soybean starch will contribute to the understanding of structure/functional property relationships for starches from all botanical sources.

Further benefit is gained because this study investigates starch characteristics of immature soybeans, which is at the developmental stage that green soybeans are consumed, known as edamame. Boiled edamame, served in or out of their pods, are typically consumed as snacks, soups, salads or vegetable dishes in Asia, and are gaining interest in USA. Quality preferences of edamame have recently been studied [11–13], and it is likely that starch contributes to overall sensory properties of edamame.

2 Materials and Methods

2.1 Plant material

High-protein (HP204), lipoxygenase-free (IA 2025, triple null, lacking all three lipoxygenase isozymes) and low-linolenic acid (IA 2064) soybean (*Glycine max* (L.) Merr.) varieties were cultivated near Boyden, Iowa (field geographic location 43° 2' 48" N, 95° 59' 36" W). Soybean seeds were obtained from the Committee for Agricultural Development, Iowa State University, Ames, IA. Soybean plots consisted of 24 rows wide and about 100 m long for each variety. Normal crop husbandry was conducted. Soybean pods estimated, based on local grower knowledge, to be approximately 20 d prior to commercial harvest were harvested on September 8, 2004, for the three varieties. Three replicates were collected corresponding to soybean pods harvested from three adjacent rows in the east, west or center of field plot. Soybean pods were transported and stored at refrigerated temperatures prior to starch extraction ($<$ 2 d).

2.2 Methods

2.2.1 Starch isolation, starch content, water, protein and oil content of soybean seeds

Starch was isolated from soybean pods using a method reported by *Kasemsuwan* et al*.* [14] with further modification by *Stevenson* [15]. One day after harvest, soybean pods were blended in 0.3% (w/v) sodium metabisulfite using a Waring commercial blender (Waring Corp., New Hartford, CT, high mode used). Soybean seeds and pods were separated and were extracted separately for starch, but no significant starch content was found in pods. Therefore seeds were not removed from pods for starch extraction. Soybean seed puree was then filtered through a screen of 106 μm mesh and the filtrate was spun at 10,500 $\times g$ for 40 min to deposit starch. To remove protein, lipids and chlorophyll, the starch pellet was washed with 10% toluene in 0.1 M aqueous sodium chloride and left standing for starch to settle out. The supernatant was decanted. This step was repeated 15–25 times. The toluene/salt solution treated starch was then washed three times with deionized water, twice with ethanol and then recovered by filtration using Whatman No. 4 filter paper. The purified starch cake was dried in a convection oven at 35°C for 48 h. Water content of soybean seeds was determined by freeze-drying finely diced seeds. Total starch content of freeze-dried, finely ground soybean seed powders, measured in duplicate for each replicate, was determined using a total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on AOAC method 996.11, AACC method 76.13 and ICC standard method No. 168, in which ground soybean seed powders are hydrolyzed with α-amylase and amyloglucosidase, and subsequent glucose content determined using glucose oxidase-peroxidase. An internal standard of corn starch was added to the samples to check quantitative recovery of starch. Oil content of freeze-dried, ground soybean seeds collected 20 d prior to harvest for each replicate was determined using a Soxtec System HT 1043 extraction unit (Tecator Inc., Herndon, VA). Protein content of freeze-dried, ground soybean seeds collected 20 d prior to harvest for each replicate was determined using the Leco® CHN-2000 series Elemental Analyzer (Leco Corp., St Joseph, MI). Compositional analysis of soybeans at harvest was measured using a near infra-red analyser (Shimadzu Scientific Instruments, Columbia, MD).

2.2.2 Starch granule morphology

Starch granules of each variety were spread on silver tape and mounted on a brass disk, then coated with gold/palladium (60/40). Sample images were observed at 1500 $\times$, 2500 $\times$ and 5000 $\times$ magnification under a scanning electron microscope (JOEL model 1850, Tokyo, Japan).

2.2.3 Starch crystallinity

Crystallinity of starch granules was determined using X-ray diffractometry. X-ray diffraction patterns were obtained with copper K_α radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following the procedure reported by *Song* and *Jane* [16]. Percentage crystallinity was calculated following the method of *Hayakawa* et al*.* [17]. The following equation was used to determine percentage crystallinity:

Crystallinity $(\%) = A_C/(A_c + A_a) \times 100$

where A_c = crystalline area and A_a = amorphous area, on the X-ray diffractogram.

2.2.4 Molar mass and gyration radius of amylopectin

Weight-average molar mass and *z*-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch analysis, with duplicate measurements of each replicate for all varieties, was conducted following a method reported by *Yoo* and *Jane* [18]. The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, a Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., JM Science, Grand Island, NY) were used following methods of *Yoo* and *Jane* [19], except from flow rate being 0.6 mL/min and sample concentration 0.5 mg/mL.

2.2.5 Apparent and absolute amylose contents

Apparent and absolute amylose contents of starch were determined by measuring iodine affinities of defatted whole starch and that of the amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY) [20]. Starch samples were defatted by dissolving in 90% dimethyl sulfoxide (DMSO) solution and followed by alcohol precipitation. The analysis was duplicated for each replicate of each soybean variety.

2.2.6 Amylopectin branch chain-length distribution

Amylopectin was separated by selective precipitation of amylose using *n*-butanol as a complexing agent [21]. Amylopectin (2 mg/mL) was defatted in 90% DMSO at 100°C for 1 h, followed by stirring at 25°C for 24 h and then dispersed in 0.1 M aqueous sodium acetate, pH 4.5, and debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN 102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by *Jane* and *Chen* [22]. Branch chain-length distribution of amylopectin was determined by using an HPAEC system (Dionex-300 and Dionex-GP50 gradient pump, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (Dionex-ED50, Sunnyvale, CA) (HPAEC-ENZ-PAD) [23]. PA-100 anion exchange analytical column (250 × 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously [24] except that Chromeleon® version 6.50 software was used. HPAEC-ENZ-PAD analysis was duplicated for each replicate of each soybean variety.

2.2.7 Thermal properties of starch

Thermal properties of starch were determined by using a differential scanning calorimeter (DSC 2920 modulated, TA Instruments, New Castle, DE) [25]. Starch (2 mg, dry starch basis, dsb) was accurately weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. The sample was allowed to equilibrate for 2 h and scanned at a rate of 5°C/min over a temperature range of 0–120°C. An empty pan was used as the reference. The rate of starch retrogradation was determined using the same gelatinized samples, stored at 4°C for 7 d, and analyzed using the same process described for gelatinization [26]. All thermal analyses were conducted in triplicate for each replicate of each soybean variety.

2.2.8 Pasting properties of starch

Starch pasting properties were analyzed using a Rapid Visco Analyser (RVA-4, Foss North America, Eden Prairie, MN) [25]. Starch suspension (8%, w/w), in duplicate for each replicate of each variety, was prepared by weighing starch (2.24 g, dsb) into a RVA canister and making up the total weight to 28 g with distilled water. The starch suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at 95°C for 5.5 min, cooled to 50°C at a rate of 6.0°C/min and maintained at 50°C for 5 min. Constant paddle rotating speed (160 rpm) was used throughout the entire analysis, except for rapid stirring at 960 rpm for the first 10 s to disperse the sample.

2.2.9 Statistical analysis

All statistical significance tests were calculated using SAS [24] and applying Tukey difference test [25] at the 5% level of significance.

3 Results and Discussion

3.1 Starch content

Starch contents of soybean varieties, shown in Tab. 1, varied from 10.9% to 11.7% of dry matter, and were not significantly

Tab. 1. Seed weight as a proportion of total soybean pod weight, and water, starch, protein and oil content of soybean seeds collected 20 d prior to harvest.

Soybean variety	Seed weight [% of total pod]	Water content [%]	Starch content [% dry weight][1]	Protein content [% dry weight][1]	Oil content [% dry weight][1]
High-protein	49.3	64.0	11.7	39.5	18.0
Lipoxygenase-free	52.0	64.4	11.5	40.8	17.3
Low-linolenic acid	52.6	63.7	10.9	39.4	17.0
	$P = 0.08$[2]	$P = 0.53$	$P = 0.24$	$P = 0.54$	$P = 0.21$

[1] Starch, protein and oil contents were averaged of two analyses from each of three replicates.
[2] *P* represents the probability of F-statistic exceeding expected for each comparison between soybean varieties in the respective column.

Tab. 2. Water, protein, oil, fiber and carbohydrate content of soybean seeds collected at commercial harvest maturity. Values after ± represent standard deviation.

Soybean variety	Water [%]	Protein [%]	Oil [%]	Fiber [%]	Carbohydrate [%]
High-protein	12.3 ± 0.1	39.6 ± 0.1	17.6 ± 0.1	4.5 ± 0.0	20.3 ± 0.2
Lipoxygenase-free	11.7 ± 0.1	38.6 ± 0.1	17.8 ± 0.2	4.7 ± 0.0	20.8 ± 0.2
Low-linolenic acid	10.8 ± 0.1	37.1 ± 0.1	18.9 ± 0.1	4.8 ± 0.0	21.2 ± 0.1

different. The starch contents we report for soybeans approximately 20 d prior to harvest was in agreement with starch content of 10–15% reported for soybeans at identical developmental stage [7]. Composition of soybean seeds is shown for 20 d prior to harvest (Tab. 1) and at commercial harvest maturity (Tab. 2).

3.2 Starch granule morphology

Scanning electron micrographs showed that three soybean starches had very small spherical to polyhedral granules, with diameters ranging between 0.7 to 4 μm (Fig. 1). Soybean starch has some of the smallest granules observed, with similar diameters compared with Chinese taro, small pigweed and parsnip starch, but not quite as small as Iliuaua dasheen, amaranth and cow cockle starch [29]. Since mature soybeans have very little starch, the small granules with large specific surface area may allow rapid starch degradation and partitioning of carbohydrate into oil and protein. Larger barley [30] and wheat [31] starch granules were more resistant to hydrolysis by α-amylase, but *Home* et al. [32] reported no difference in α-amylase hydrolysis between small and large barley starch granules, while *Baker* and *Woo* [33] reported that larger (15–20 μm) wheat starch granules were 2.4 fold more susceptible to hydrolysis by insect α-amylase than smaller granules (5–10 μm), and α-amylase, depending on isoenzyme, was found to preferentially digest small or large granules of *Araucaria araucana* seed starch [34].

3.3 Starch crystalline structure

Soybean starches all exhibited C_B-type X-ray diffraction patterns (mixture of A- and B-type polymorphs) (Fig. 2), with a strong peak at $2\Theta = 17.2°$, characteristic of B-type crystallinity (or polymorph), but only a slight peak at $2\Theta = 5.5°$, which is smaller than typical for B-type crystallinity (polymorph). The lack of a split peak at $2\Theta = 22–24°$ and another peak at $2\Theta = 14.6°$, however, are characteristics of A-type crystallinity. The X-ray diffraction patterns indicate that soybean starches possess the C-type crystallinity with more B-type polymorphs than A-type polymorphs. The only other C-type starches reported were isolated from green banana fruit [25], lotus root [25], water chestnuts [25, 35], apple fruit [36, 37], sweet potato tubers [24, 38], sago palm [39, 40], and seeds of pea [41], pigeon pea [42], lima bean [43] and ginkgo [44]. Percentage crystallinity of high-protein, lipoxygenase-free and low-linolenic acid soybean starches, calculated based on X-ray diffractograms, was 27.7%, 36.7% and 33.3%, respectively. Since soybean starch consists of a mixture of A- and B-type polymorphs, it is not surprising that percentage crystallinity falls within the range previously reported for numerous A- and B-type starches [45].

3.4 Iodine affinity and amylose content

The iodine affinities of the defatted whole starches and the corresponding apparent amylose contents were significantly different among the soybean varieties ($P = 0.05$).

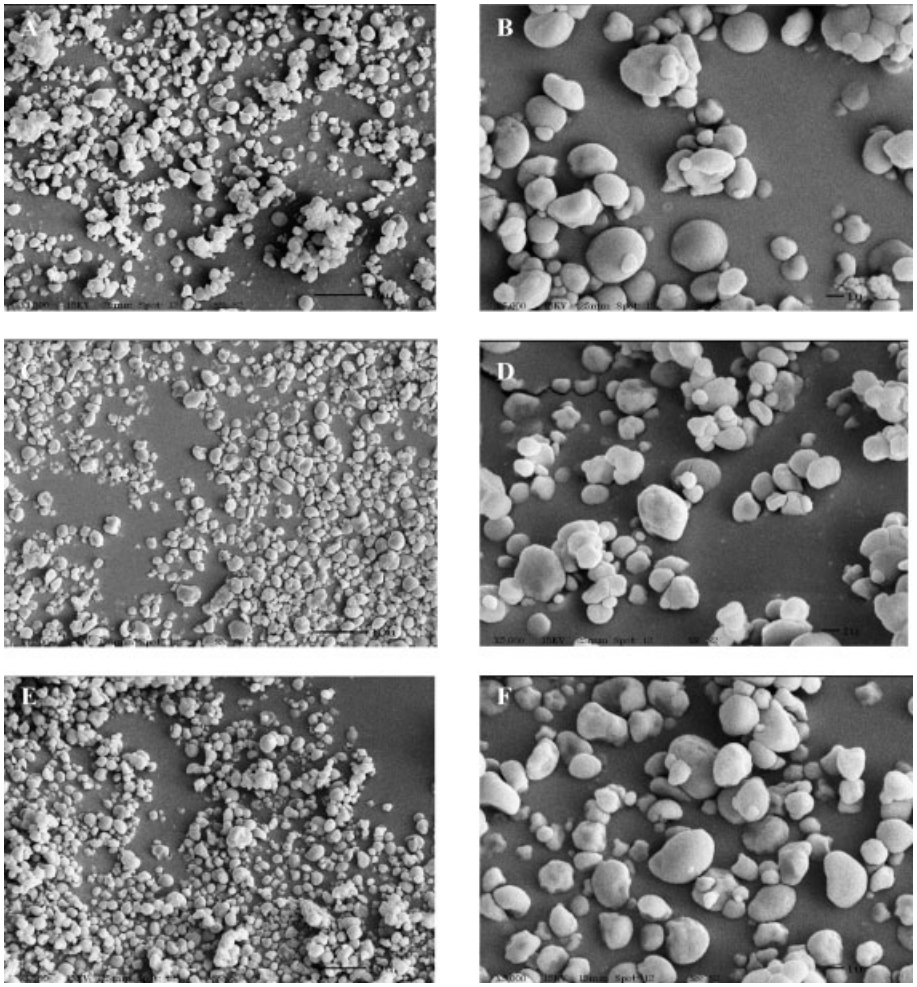

Fig. 1. Scanning electron micrographs at 1500× and 5000× magnification of high-protein (A and B), lipoxygenase-free (C and D) and low-linolenic acid (E and F) soybean seed starch. Scale bar is 10 μm for A, C and E and 1 μm for B, D and F.

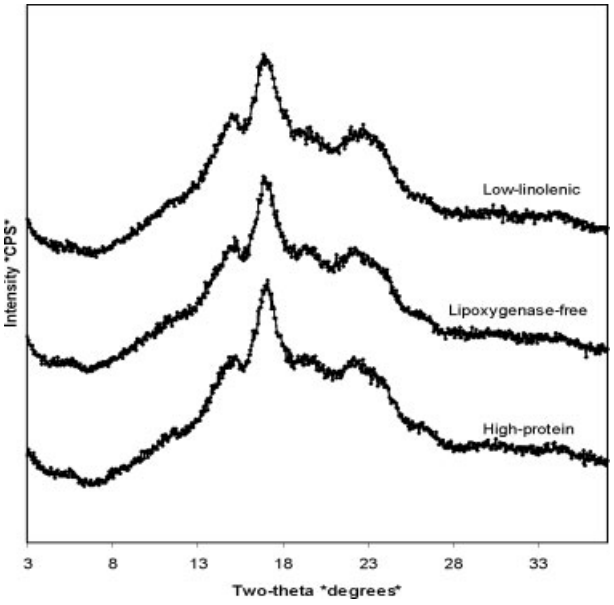

Fig. 2. X-ray diffraction patterns of high-protein, lipoxygenase-free and low-linolenic acid soybean starches.

The low-linolenic acid soybean variety had significantly higher apparent amylose content than the high-protein soybean variety ($P = 0.05$) and significantly higher absolute amylose content ($P = 0.05$) than the lipoxygenase-free soybean variety (Tab. 3). Apparent amylose contents of soybean starches are lower than most other normal starches, but not uncommon [15]. Absolute amylose contents of high-protein and lipoxygenase-free soybeans are lower than that reported for any other normal starch [15], whereas absolute amylose content of low-linolenic acid soybeans is similar to Chinese taro [25], ebiimo [46] and cattail millet [25]. The low iodine affinity of soybean amylopectin fraction is typical of many A-type starches [25] and suggests amylopectin has short branch-chains. Linolenic acid, because of its oxidation propensity, is considered an undesirable component of soybean oil and our results suggest further research focusing on soybean varieties with differing fatty acid composition would be worthwhile to ascertain if amylose content is related to oil linolenic acid content or the extent of oil unsaturation.

Tab. 3. Iodine affinities, apparent amylose and absolute amylose contents of defatted soybean starches.

Soybean variety	Iodine affinity		Apparent amylose content [%][1]	Absolute amylose content [%][2]
	Whole starch	Amylopectin fraction		
High-protein	3.85b[3]	1.16	19.3b	13.9ab
Lipoxygenase-free	4.11ab	2.00	20.7ab	11.8b
Low-linolenic acid	4.35a	1.33	21.9a	16.2a
	$P = 0.05$[4]	$P = 0.07$	$P = 0.05$	$P = 0.05$

[1] Apparent amylose contents were averaged from two analyses for each of three replicates. Values were calculated from dividing iodine affinity by a factor of 0.199.
[2] Absolute amylose contents were averaged from two analyses for each of three replicates. Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.
[3] Values with different letters denote differences at the 5% level of significance for each comparison between soybean varieties in the respective column.
[4] *P* represents the probability of F-statistic exceeding expected for each comparison between soybean varieties in the respective column.

Tab. 4. Average amylopectin molecular weight, polydispersity, gyration radius and density of dispersed soybean amylopectin.[1]

Soybean variety[2]	$M_w \times 10^8$ [g/mol][3]	Polydispersity [M_w]	R_z [nm][4]	ρ [g mol^{-1}nm^{-3}][5]
High-protein	11.29	4.52	510	8.4
Lipoxygenase-free	5.59	2.09	380	10.1
Low-linolenic acid	5.11	2.93	406	7.7
	$P = 0.31$[6]	$P = 0.59$	$P = 0.45$	$P = 0.64$

[1] Data were obtained from two analyses for each of three replicates.
[2] Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol. Freshly prepared starch aqueous solution (100 μL; 0.8 mg/mL) was injected to HPSEC system.
[3] *weight*-average molar mass.
[4] *z*-average radius of gyration.
[5] Dispersed amylopectin density is equal to M_w/R_z^3. Values for density may not correspond directly to data in table due to rounding of M_w and R_z.
[6] *P* represents the probability of F-statistic exceeding expected for each comparison between soybean varieties in the respective column.

3.5 Amylopectin molecular weight and size

Weight-average molar mass (M_w), polydispersity, and gyration radius (R_z) of soybean starch amylopectins are shown in Tab. 4. The amylopectin M_w of the high-protein soybean variety was double that of the other two varieties but high variation among different field replicates meant that no significant differences could be determined. Soybean amylopectin M_w from all varieties was larger than that of lotus root and green banana, but comparable to water chestnut and apple C-type starches [19, 36]. Soybean amylopectin M_w was larger than that of all B-type starches, but comparable to most A-type starches [19], suggesting that there may be more A-polymorphs present than appears from X-ray diffraction patterns (Fig. 2). The polydispersities (M_w/M_n) of soybean amylopectin molar mass were similar to other A-type starches [15].

R_z values of soybean amylopectin were larger than those reported for all other C-type amylopectins except from apple starch [19, 36]. High-protein soybean variety amylopectin R_z of 510 nm was higher than all other starches except for Chinese taro, normal rice and waxy rice [19]. Soybean amylopectin density was low relative to most starches, but was comparable with C-type lotus root and green banana starch [19].

3.6 Amylopectin branch chain-length distribution

Amylopectin branch chain-length distributions of the soybean varieties are shown in Fig. 3 and summarized in Tab. 5. There were no significant differences among soybean varieties. The most distinctive characteristics of all soybean

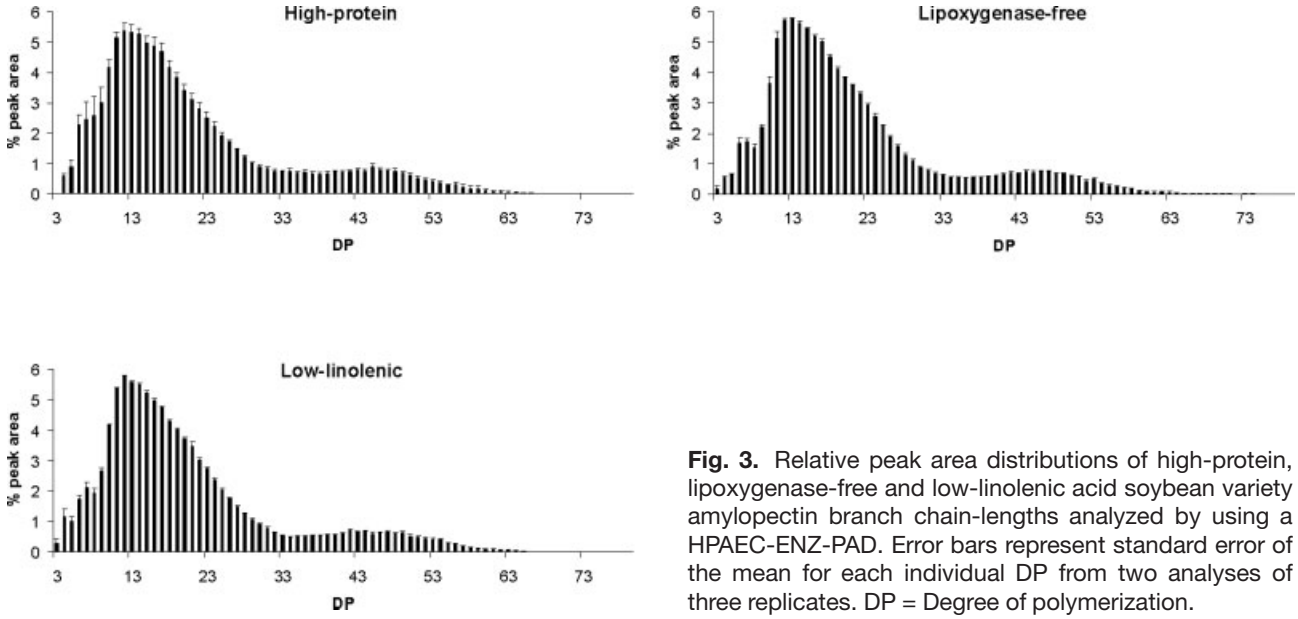

Fig. 3. Relative peak area distributions of high-protein, lipoxygenase-free and low-linolenic acid soybean variety amylopectin branch chain-lengths analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of three replicates. DP = Degree of polymerization.

Tab. 5. Branch chain-length distributions of soybean seed amylopectins[1,2].

Soybean variety	Peak DP		Average CL	Percent distribution					
	I	II		DP 3–5	DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP $\geq$ 37
High-protein	12.3	47.8	20.8	1.5	10.3	25.0	47.2	12.7	13.4
Lipoxygenase-free	12.7	46.3	20.9	1.3	7.1	21.6	51.9	12.7	12.3
Low-linolenic acid	12.0	46.3	20.4	2.4	8.5	23.8	49.7	12.1	11.7
			$P = 0.56$[3]	$P = 0.25$	$P = 0.48$	$P = 0.37$	$P = 0.40$	$P = 0.64$	$P = 0.36$

[1] Grouping of degree of polymerization (DP) numbers followed that of *Hanashiro* et al. [71] except DP 3–5 and 6–9.
[2] Values were calculated from two injections for each of three replicates.
[3] *P* represents the probability of F-statistic exceeding expected for each comparison between soybean varieties in the respective column.

amylopectins were the very low proportion (11.7–13.4%) of long branch-chains (DP $\geq$ 37) and the short average branch chain-length (Tab. 5, Fig. 3). Only the branch chain-length distribution of waxy rice amylopectin has been reported to have a lower proportion of long branch-chains (DP $\geq$ 37) and average branch chain-length [25]. The short amylopectin branch-chains of soybean starch agreed with the observed low iodine affinity of the amylopectin fraction. Since A-type starches have considerably shorter amylopectin branch-chains than B-type starches [25], soybean amylopectin branch chain-length distribution also suggests that more A-type polymorphs are present in the starch than indicated by X-ray diffraction patterns (Fig. 2). The short average amylopectin branch chain-length observed for soybean starches considerably contrasted reports for other C-type starches such as lotus root, water chestnut and green banana (25.4–26.7) [25], and the very long chains of apple (27.9–29.6) [36]. Since soybean starches were extracted from seeds 20 d prior to harvest, and soybeans have $<$ 1% starch at harvest [5], the short amylopectin branch chain-length distribution may create favorable structural architecture for rapid amylopectin degradation. Faster hydrolysis rate by amylases digesting shorter amylopectin branch-chains has previously been reported [44, 47–51].

Because starch granules were very irregular, and both amylopectin M_w and branch chain-length distribution were typical of starches with A-type polymorphs, it is possible that soybean starches 20 d prior to harvest could have experienced partial amylolytic hydrolysis. Soybeans contain β-amylase [2, 52, 53] that may have hydrolyzed starch granules, but starch metabolism was found to be independent of β-amylase activity in developing soybean seeds [54]. Starch damaged by enzymatic attack is known to retrograde and form B-type polymorphs [55–59]. Therefore it is possible that the C_B-type crystallinity X-ray diffraction pat-

Tab. 6. Thermal properties of native soybean starches.

Soybean variety[1]	Starch gelatinization			Amylose-lipid thermal transition		
	T_o [°C][2]	T_p [°C]	ΔH [J/g]	T_o [°C]	T_p [°C]	ΔH [J/g]
High-protein	52.6	56.8	12.9	87.6	91.5	1.52
Lipoxygenase-free	53.5	57.9	12.6	86.8	91.8	1.86
Low-linolenic acid	52.0	56.5	12.3	87.4	91.3	1.67
	$P = 0.47$[3]	$P = 0.48$	$P = 0.88$	$P = 0.37$	$P = 0.17$	$P = 0.73$

1 Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, and ΔH are onset and peak temperature, and enthalpy change of gelatinization, respectively.
2 Values were calculated from three analyses for each of three replicates.
3 *P* represents the probability of F-statistic exceeding expected for each comparison between soybean varieties in the respective column.

Tab. 7. Thermal properties of retrograded soybean starch.

Soybean variety[1]	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]	% Retrogradation
High-protein	36.9	53.3	64.3	7.1	41.6
Lipoxygenase-free	36.1	52.4	63.3	7.4	42.3
Low-linolenic acid	37.5	53.4	64.5	7.1	45.2
	$P = 0.34$[2]	$P = 0.20$	$P = 0.21$	$P = 0.88$	$P = 0.95$

1 Same starch samples after gelatinization (see Tab. 5) were stored for 7 days at 4°C and rescanned using DSC.
2 *P* represents the probability of F-statistic exceeding expected for each comparison between soybean varieties in the respective column.

Tab. 8. Pasting properties of soybean variety starches measured by Rapid Visco Analyser[1,2].

Soybean variety	Peak viscosity [RVU]	Breakdown [RVU]	Final viscosity [RVU]	Setback [RVU]	Pasting temperature [°C]
High-protein	81.0	9.9	100.3	29.3	71.6
Lipoxygenase-free	81.6	10.0	92.6	21.0	83.6
Low-linolenic acid	93.0	23.2	106.2	36.4	83.8
	$P = 0.85$[3]	$P = 0.99$	$P = 0.80$	$P = 0.54$	$P = 0.51$

1 8% (w/w) starch suspension measured in duplicate for all three replicates.
2 Viscosity measured in Rapid Visco-Analyser units (RVU), 1 RVU = 12 mPas.
3 *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

tern we observed actually indicates that soybean starch has A-type crystallinity and is partially hydrolyzed to form B-type polymorphs at edamame development stage.

3.7 Thermal properties

Thermal properties of the soybean starches are shown in Tab. 6. No significant differences were observed in starch gelatinization or melting of amylose-lipid complex. Onset gelatinization temperatures (T_o) for all soybean starches were relatively low compared with all other starches [15] with only starch from a few cultivars or varieties of arrowroot [46], barley [60], buckwheat [61], oat [62], sweet potato [63] and wheat [46, 64] being lower. The low T_o of soybean starch is most likely due to the short amylopectin branch-chains, because a positive relationship between T_o and amylopectin branch chain-length has been reported [25, 60]. Thermal properties of retrograded soybean starches were similar for all three varieties (Tab. 7).

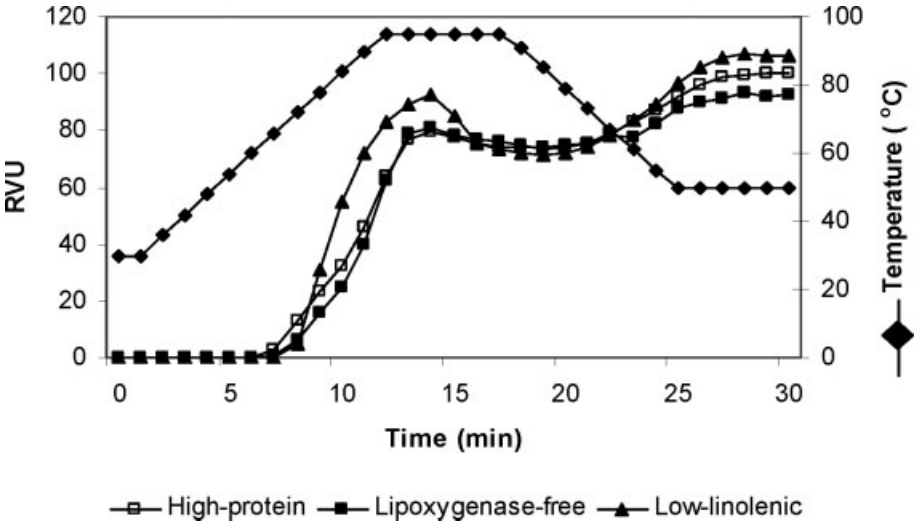

Fig. 4. Rapid Visco Analyser pasting profiles of high-protein, lipoxygenase-free and low-linolenic acid soybean starches (8.0% dsb, w/w).

3.8 Pasting properties

Pasting properties of soybean starches are shown in Fig. 4 and Tab. 8. Large variation in pasting properties among the field replicates for each variety meant that no significant differences in pasting parameters among the varieties were observed. Relative to an 8% (w/w) suspension of other starches, soybean starch had considerably low peak and breakdown viscosity, and moderately low final and setback viscosity [25]. The low peak viscosity of soybean starch could be due to short amylopectin branch-chains, which has previously been correlated in wheat starch [65, 66]. However, peak viscosity negatively correlated to proportion of amylopectin chains of length DP $\geq$ 37 has also been reported [60, 67]. Soybean starch amylose content probably did not contribute greatly to low peak viscosity, because higher apparent amylose content has been reported to result in lower peak viscosity [25, 68, 69]. Low breakdown viscosity is surprising considering soybean starch has short amylopectin branch-chains that favor starch paste breakdown under shear [70].

4 Conclusion

Three soybean varieties (high-protein, lipoxygenase-free and low-linolenic acid collected 20 d prior to harvest) had very small starch granules ranging 0.7–4 μm, and starches exhibited C_B-type X-ray diffraction patterns. Apparent amylose content ranged from 19–22%, absolute amylose content was very low, ranging from 12–16% and amylopectin molar mass ranged from 0.5–1.1 $\times$ 10^9 g/mol. Average amylopectin branch chain-length was very short (20.4–20.9) that may enable rapid starch degradation in late stages of maturity. Despite the abundance of more B-type polymorphs, soybean starch structure had greater similarities with A-type polymorphs which may reflect that soybeans at edamame stage experience partial starch hydrolysis from β-amylase. Future studies investigating changes in starch structure during development of soybean seeds would contribute useful information. Peak (81–93 RVU) and final (93–106 RVU) paste viscosity were very low. Onset gelatinization temperature was 52–54°C and ΔH was (12–13 J/g). Low-linolenic acid soybeans had higher apparent amylose content than high-protein soybeans and higher absolute amylose content than lipoxygenase soybeans, suggesting that possibly higher amylose contents of starch during development may reduce the levels of undesirable linolenic acid in mature soybeans. Studies investigating whether soybeans with different fatty acid oil composition have different starch structures would be worthwhile. A high variation among field replicates was observed for starch characteristics of each soybean variety, with additional differences in starch structures and functional properties of soybean varieties likely to be found upon better understanding of sources of variation.

Acknowledgements

We thank *Arthur Thompson* for scanning electron microscopy assistance, *Scott Schlorholtz* for X-ray diffraction assistance and *Amy Lin* for assistance with amylopectin branch chain-length analysis.

References

[1] American Soybean Association: Soy stats™ 2004. A reference guide to important soybean facts and figures. American Soybean Association, Saint Louis, MO, **2004**.

[2] D. F. Hildebrand, T. Hymowitz: Role of β-amylase in starch metabolism during soybean seed development and germination. *Physiologia Plantarum* **1981**, *53*, 429–434.

[3] V. Pöysä, L. Woodrow: Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. *Food Res. Int.* **2002**, *35*, 337–345.

[4] J. R. Wilcox, R. M. Shibles: Interrelationships among seed quality attributes in soybean. *Crop Sci.* **2001**, *41*, 11–14.

[5] L. A. Wilson, V. A. Birmingham, D. P. Moon, H. E. Snyder: Isolation and characterization of starch from mature soybeans. *Cereal Chem.* **1978**, *55*, 661–670.

[6] T. G. Isleib, H. E., Pattee, F. G. Giesbrecht: Oil, sugar, and starch characteristics in peanut breeding lines selected for low and high oil content and their combining ability. *J. Agric. Food Chem.* **2004**, *52*, 3165–3168.

[7] B. Yazdi-Samadi, R. W. Rinnie, R. D. Seif: Components of developing soybean seeds: oil protein, sugars, starch, organic acids, and amino acids. *Agronomy J.* **1977**, *69*, 481–486.

[8] J. M. G. Thomas, K. J. Boote, L. H. Allen, M. Gallo-Meagher, J. M. Davis: Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance. *Crop Sci.* **2003**, *43*, 1548–1557.

[9] S. D. Golombek, R. Sridhar, U. Singh: Effect of soil temperature on the seed composition of three Spanish cultivars of groundnut (*Arachis hypogaea* L.). *J. Agric. Food Chem.* **1995**, *43*, 2067–2070.

[10] M. Nakamura: Structure of starch granules. II. [beans, barley, soybeans]. *Denpun Kagaku – J. Jap. Soc. Starch Sci.* **1974**, *21*, 230–254.

[11] K. M. Kelley, E. S. Sánchez: Accessing and understanding consumer awareness of and potential demand for edamame. *HortSci.* **2005**, *40*, 1347–1353.

[12] A. L. Wszelaki, J. F. Delwiche, S. D. Walker, R. E. Liggett, S. A. Miller, M. D. Kleinhenz: Consumer liking and descriptive analysis of six varieties of organically grown edamame-type soybean. *Food Qual. Pref.* **2005**, *16*, 651–658.

[13] T. Akazawa, E. Shiraiwa, N. Satoh, T. Sasahara: Variations in eating quality, flavour and pod colour of green soybeans from vegetable-type and grain-type plants. *Jap. J. Crop Sci.* **2002**, *71*, 62–67.

[14] T. Kasemsuwan, J. Jane, P. Schnable, P. Stinar, D. Robertson: Characterization of the dominant mutant amylose-extender (*Ae*1–5180) maize starch. *Cereal Chem.* **1995**, *71*, 457–464.

[15] D. G. Stevenson: Role of starch structure in texture of winter squash (Cucurbita maxima D.) fruit and starch functional properties, (pp. 18–24, 48–53, 158, 173–174). *PhD Thesis*, Iowa State University, Ames, IA, **2003**.

[16] Y. Song, J. Jane: Characterization of barley starches of waxy, normal, and high amylose varieties. *Carbohydr. Polym.* **2000**, *41*, 365–377.

[17] K. Hayakawa, K. Tanaka, T. Nakamura, S. Endo, T. Hoshino: Quality characteristics of waxy hexaploid wheat (*Triticum aestivum* L.): Properties of starch gelatinization and retrogradation. *Cereal Chem.* **1997**, *74*, 576–580.

[18] S. Yoo, J. Jane: Structural and physical characteristics of waxy and other wheat starches. *Carbohydr. Polym.* **2002**, *49*, 297–305.

[19] S. Yoo, J. Jane: Molecular weights and gyration radii of amylopectins determined by high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors. *Carbohydr. Polym.* **2002**, *49*, 307–314.

[20] T. Lu, J. Jane, P. L. Keeling, G. W. Singletary: Maize starch fine structures affected by ear developmental temperature. *Carbohydr. Res.* **1996**, *282*, 157–170.

[21] T. J. Schoch: Fractionation of starch by selective precipitation with butanol. *J. Am. Chem. Soc.* **1942**, *64*, 2957–2961.

[22] J. Jane, J. F. Chen: Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* **1992**, *69*, 60–65.

[23] K. S. Wong, J. Jane: Quantitative analysis of debranched amylopectin by HPAEC-PAD with a post-column enzyme reactor. *J. Liq. Chromatogr.* **1997**, *20*, 297–310.

[24] A. E. McPherson, J. Jane: Comparison of waxy potato with other root and tuber starches. *Carbohydr. Polym.* **1999**, *40*, 57–70.

[25] J. Jane, Y. Y. Chen, L. F., Lee, A. E., McPherson, K. S. Wong, M. Radosavljevic, T. Kasemsuwan: Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.* **1999**, *76*, 629–637.

[26] P. J. White, I. R. Abbas, L. A. Johnson: Freeze-thaw stability and refrigerated-storage retrogradation of starches. *Starch/Stärke* **1989**, *41*, 176–180.

[27] SAS Institute Inc.: The SAS® system for Windows®, version 8e. Cary, NC, **1999**.

[28] F. L. Ramsey, D. W. Schafer: *The statistical sleuth: A course in methods of data analysis (pp. 154)*. Belmont, CA: Duxbury Press, **1996**.

[29] J. Jane, T. Kasemsuwan, S. Leas, H. Zobel, J. F. Robyt: Anthology of starch granule morphology by scanning electron microscopy. *Starch/Stärke* **1994**, *46*, 121–129.

[30] A. W. MacGregor, D. L. Ballance: Hydrolysis of large and small granules from normal and waxy barley cultivars by alpha-amylases from barley malt. *Cereal Chem.* **1980**, *57*, 397–402.

[31] T.-J. Lu, Y.-Y. Pai, C.-Y. Lii: The susceptibility of A- and B-type wheat starch granules to bacterial alpha-amylase. *Proc. American Assoc. Cereal Chem. Annual Meeting*, Seattle, WA, Oct 31- Nov 3, **1999**, abstract 241.

[32] S. Home, K. Stenholm, A. Wilhelmson, K. Autio: Properties of starch and cell wall components and their effects on processing. *Proc. 9th Australian Barley Technical Symposium*, Melbourne, Australia, September 12–16, **1999**, pp. 2.37.1–2.37.8.

[33] J. E. Baker, S. M. Woo: Digestion of starch granules by alpha-amylases from the rice weevil, *Sitophilus oryzae*: effect of starch type, fat extraction, granule size, mechanical damage, and detergent treatment. *Insect Biochem. Mol. Biol.* **1992**, *22*, 529–537.

[34] J. J. Waghorn, T. del Pozo, E. A. Acevedo, L. A. Cardemil: The role of two isoenzymes of α-amylase of *Araucaria araucana* (Araucariaceae) on the digestion of starch granules during germination. *J. Exp. Bot.* **2003**, *54*, 901–911.

[35] V. Tulyathan, K. Boondee, T. Mahawanich: Characteristics of starch from water chestnut (*Trapa bispinosa Roxb.*). *J. Food Biochem.* **2005**, *29*, 337–348.

[36] D. G. Stevenson, P. A. Domoto, J. Jane: Structures and functional properties of apple (*Malus domestica* Borkh) fruit starch. *Carbohydr. Polym.* **2006**, *63*, 432–441.

[37] N. Singh, N. Inouchi, K. Nishinari: Morphological, structural, thermal, and rheological characteristics of starches separated from apples of different cultivars. *J. Agric. Food Chem.* **2005**, *53*, 10193–10199.

[38] N. K. Genkina, L. A. Wasserman, T. Noda, R. F. Tester, V. P. Yuryev: Effects of annealing on the polymorphic structure of starches from sweet potatoes (Ayamurasaki and Sunnyred cultivars) grown at various soil temperatures. *Carbohydr. Res.* **2004**, *28*, 1093–1098.

[39] F. B. Ahmad, P. A. Williams, J. L. Doublier, S. Durand, A. Buléon: Physico-chemical characterisation of sago starch. *Carbohydr. Polym.* **1999**, *38*, 361–370.

[40] S. Gorinstein, C. G. Oates, S. M. Chang, C.-Y. Lii: Enzymatic hydrolysis of sago starch. *Food Chem.* **1994**, *49*, 411–417.

[41] T. Y. Bogracheva, V. J. Morris, S. G. Ring, C. L. Hedley: The granular structure of C-type pea starch and its role in gelatinization. *Biopolymers* **1998**, *45*, 323–332.

[42] R. Hoover, G. Swamidas, T. Vasanthan: Studies on the physicochemical properties of native, defatted, and heat-moisture treated pigeon pea (*Cajanus cajan* L) starch. *Carbohydr. Res.* **1993**, *246*, 185–203.

[43] R. Hoover, S. C. Rorke, A. M. Martin: Isolation and characterization of lima bean (*Phaseolus lunatus*) starch. *J. Food Biochem.* **1991**, *15*, 117–136.

[44] K. E. Spence, J. Jane: Chemical and physical properties of ginkgo (*Ginkgo biloba*) starch. *Carbohydr. Polym.* **1999**, *40*, 261–269.

[45] D. Cooke, M. J. Gidley: Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydr. Res.* **1992**, *227*, 103–112.

[46] A. Suzuki: Cooking aspects of characteristics and utilization of starches. *Denpun Kagaku – J. Jap. Soc. Starch Sci.* **1993**, *40*, 233–243.

[47] W. S. Ratnayake, R. Hoover, F. Shahidi, C. Perera, J. Jane: Composition, molecular structure, and physicochemical properties of starches from four field pea (*Pisum sativum* L.) cultivars. *Food Chem.* **2001**, *74*, 189–202.

[48] Q. Zhu, E. Bertoft: Composition and structural analysis of alpha-dextrins from potato amylopectin. *Carbohydr. Res.* **1996**, *288*, 155–174.

[49] S. Govindasamy, C. G. Oates, H. A. Wong: Characterization of changes of sago starch components during hydrolysis by a thermostable alpha-amylase. *Carbohydr. Polym.* **1994**, *18*, 89–100.

[50] A. K. Mazur, H. Nakatani: Multiple attack mechanism in the porcine pancreatic alpha-amylase hydrolysis of amylose and amylopectin. *Arch. Biochem. Biophys.* **1993**, *306*, 29–38.

[51] E. Bertoft: Investigation of the fine structure of alpha-dextrins derived from amylopectin and their relation to the structure of waxy-maize starch. *Carbohydr. Res.* **1991**, *212*, 229–244.

[52] Y. Morita, F. Yagi: Characterization of beta-amylase isozymes in soybean seeds by isoelectric focusing. *Plant Cell Physiol.* **1979**, *20*, 797–802.

[53] H. Ren, J. F. Thompson, J. T. Madison: Biochemical and physiological studies of soybean beta-amylase. *Phytochem.* **1993**, *33*, 541–545.

[54] C. A. Adams, T. H. Broman, R. W. Rinne: Starch metabolism in developing and germinating soya bean seeds is independent of beta-amylase activity. *Ann. Botany* **1993**, *48*, 433–439.

[55] V. Planchot, P. Colonna, A. Buléon: Enzymatic hydrolysis of alpha-glucan crystallites. *Carbohydr. Res.* **1997**, *298*, 319–326.

[56] M. J. Gidley, D. Cooke, A. H. Darke, R. A. Hoffmann, A. L. Russell, P. Greenwell: Molecular order and structure in enzyme-resistant retrograded starch. *Carbohydr. Polym.* **1995**, *28*, 23–31.

[57] C. Hoebler, A. Karinthi, H. Chiron, M. Champ, J. L. Barry: Bioavailability of starch in bread rich in amylose: metabolic responses in healthy subjects and starch structure. *Eur. J. Clin. Nutr.* **1999**, *53*, 360–366.

[58] C. Gérard, P. Colonna, B. Bouchet, D. J. Gallant, V. Planchot: A multi-stages biosynthetic pathway in starch granules revealed by the ultrastructure of maize mutant starches. *J. Cereal Sci.* **2001**, *34*, 61–71.

[59] C. Gérard, P. Colonna, A. Buléon, V. Planchot: Amylolysis of maize mutant starches. *J. Sci. Food Agric.* **2001**, *81*, 1281–1287.

[60] J. H. Li, T. Vasanthan, B. Rossnagel, R. Hoover: Starch from hull-less barley: II. Thermal, rheological and acid hydrolysis characteristics. *Food Chem.* **2001**, *74*, 407–415.

[61] T. Noda, Y. Takahata, T. Sato, I. Suda, T. Morishita, K. Ishiguro, O. Yamakawa: Relationships between chain length distribution of amylopectin and gelatinization properties within the same botanical origin for sweet potato and buckwheat. *Carbohydr. Polym.* **1998**, *37*, 153–158.

[62] R. F. Tester, J. Karkalas: Swelling and gelatinization of oat starches. *Cereal Chem.* **1996**, *73*, 271–277.

[63] K. Katayama, K. Komae, K. Kohyama, T. Kato, S. Tamiya, K. Komaki: New sweet potato line having low gelatinization temperature and altered starch structure. *Starch/Stärke* **2002**, *54*, 51–57.

[64] T. Demeke, P. Hucl, E. S. M. Abdel-Aal, M. Båga, R. N. Chibbar: Biochemical characterization of the wheat waxy A protein and its effect on starch properties. *Cereal Chem.* **1999**, *76*, 694–698.

[65] T. Sasaki, J. Matsuki: Effect of wheat structure on swelling power. *Cereal Chem.* **1998**, *75*, 385–391.

[66] Y. Shibanuma, Y. Takeda, S. Hizukuri: Molecular and pasting properties of some wheat starches. *Carbohydr. Polym.* **1996**, *29*, 253–261.

[67] X. Z. Han, B. R. Hamaker: Amylopectin fine structure and rice starch paste breakdown. *J. Cereal Sci.* **2001**, *34*, 279–284.

[68] M. Kuno, K. Kainuma, S. Takahashi: Physicochemical characteristics of low-amylose rice starches. *J. Appl. Glycosci.* **2000**, *47*, 319–326.

[69] Y. Wang, P. White, L. Pollak: Physicochemical properties of starches from mutant genotypes of the Oh43 inbred line. *Cereal Chem.* **1993**, *70*, 199–203.

[70] K. R. Reddy, S. Z. Ali, K. R. Bhattacharya: The fine structure of rice-starch amylopectin and its relation to the texture of cooked rice. *Carbohydr. Polym.* **1993**, *22*, 267–275.

[71] I. Hanashiro, J. Abe, S. Hizukuri: A periodic distribution of the chain length of amylopectin revealed by high-performance anion-exchange chromatography. *Carbohydr. Res.* **1996**, *283*, 151–159.

(Received: June 15, 2006)
(Accepted: August 7, 2006)

